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ENHANCED ANTI INFLAMMATORY ACTIVITY OF PHYTO-STABILIZED SILVER NANOPARTICLES ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

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ABSTRACT

Phytostabilized silver nanoparticles are being widely studied for its various pharmacological properties. In the present study silver nanoparticles synthesized using Black pepper extract was evaluated for its acute toxicity and also for its anti inflammatory activity in carrageenan induced paw edema in rats. The anti inflammatory effect was also compared against the commercial silver nanoparticles. The phyto-stabilised silver nanoparticles did not show any signs of toxicity at 300mg/kg given by intraperitoneal route. In the carrageenan induced paw edema model, the phytostabilised silver nanoparticles at 50mg/kg showed significant inhibition of paw edema at 2,3 and 5 hrs with a maximum of 62.50 % inhibition at the end of 5 hrs, thus exhibiting enhanced activity when compared to the commercial silver nanoparticles due to the additional properties of the capping phytochemicals.

KEYWORDS: Phyto-stabilised silver nanoparticles, acute toxicity, anti-inflammatory, Carrageenan model

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INTRODUCTION

Inflammation is a self protective response of the body to remove any harmful stimuli. It is a complex process involving various cellular components and inflammatory mediators[1]. Though a protective phenomena, inflammation can cause damage to the host tissue and is a common pathology underlying various diseases including diabetes and cancer. The conventional anti inflammatory drugs lack efficacy and also produce various deleterious effects[2]. With the advent of newer technologies, researchers are striving hard to discover newer and potent anti inflammatory drugs. Nanotechnology is an exciting approach for the development of novel anti inflammatory drugs. Silver nanoparticles are known to possess wound healing and anti inflammatory properties[3]. Green synthesis of silver nanoparticles using phytochemicals as capping and stabilizing agents have gained supremacy as it is non toxic and economical. Moreover, the phyto stabilized silver nanoparticles also have enhanced biological activities[4]. In our previous study we have reported the facile synthesis of phyto stabilized silver nanoparticles using *Piper nigrum* extract. *Piper nigrum* commonly referred to as Black pepper is a commonly used spice and also as a traditional medicine for joint pains and toothache[5]. Carrageenan induced rat paw edema is the most widely used model to study the acute and sub acute phases of inflammation in rodents. Carrageenan is a phlogistic agent which initiates a cascade of events leading to formation of exudates. The inflammation induced is typically biphasic. The first phase is attributed to the release of histamine, 5HT and kinins, while the second phase is related to the release of prostaglandins[6]. The current study was done to evaluate the anti inflammatory activity of phytostabilised silver nanoparticles on carrageenan induced paw edema and to compare the effect with chemically synthesized silver nanoparticles.

MATERIALS AND METHODS

1. Synthesis and Characterization of Phyto-stabilized silver nanoparticles

Silver nanoparticles were synthesized using the aqeous extract of black pepper, and was characterized using UV-spectrophotometer, FTIR and SEM which was published in our previous study[7]. In brief, Black pepper extract was prepared by decoction method. To 90ml of 1mMol AgNO3 solution , 10 ml of the extract was added and kept on a magnetic stirrer for 2 hrs.

2. Pharmacological & Toxicological Tests

2.1. Animals

Albino wistar rats, weighing about 150-200 mg were purchased from BIOGEN, Bangalore. Animals were housed in a polypropylene cage with good ventilation and room temperature 22°C (±3°) and relative humidity 53–60% were maintained. Animals had free access to pelleted feed (M/s. Provimi Animal Nutrition Pvt. Ltd, India) and Reverse osmosis (Rios, USA) purified water *ad libitum*. All animals were acclimatized for five days before the start of the experimental session. The Institutional Animal Ethical Committee clearance was obtained (IAEC No: IAEC/XXXIV/SRU/290/2013) and the study was done following the guidelines of the Institutional Animals Ethics Committee (IEAC).

2.2. Chemicals

Carrageenan and commercial silver nanoparticles(Chemically synthesized) were purchased from Sigma Aldrich Co.

2.3. Acute toxicity test

Acute intraperitoneal toxicity study was performed using healthy young adult nulliparous and non-pregnant Albino Wistar rats. This experiment was conducted with step wise procedure. At each step 300 mg/kg b.wt was given by intraperitoneal route to three animals. Lethality and abnormal clinical signs were observed. Clinical signs were observed on the day of dosing at 30 min after 1hr, 2hr and 4hr and upto14 days. Body weights were recorded just prior to dosing and thereafter once a week till completion of the experiment.

Gross pathological changes were also observed at the end of observation period.

2.4. Carrageenan Induced Paw edema in Rats

Animals were divided into six groups each having 6 animals each. Group A (carrageenan control) did not receive treatment; Group B (standard) received Diclofenac Sodium (25mg./kg), Group C and Group D received synthesized silver nanoparticles (SNPn) at 50mg/kg and 100mg/kg body weight and Group E animals were administered commercial silver nanoparticles synthesized using standard chemical method (CNP) at 100mg/kg body weight). All doses were administered by the intraperitoneal route. Edema was induced by injecting 0.1 ml of 1% freshly prepared carrageenan solution into the sub plantar region of right-hind paws of each rat of all the groups. Basal paw volumes were measured using a Plethysmometer. The test drugs were administered 30 mins prior to the carrageenan injection and the measurement of paw volumes were done just before the carrageenan injection, that is, at “0 hour” and then at 30 mins, 1, 2, 3, and 5th hour after carrageenan injection using a plethysmometer. Increase in paw volume was measured as the difference in paw volume at “0 hour” and paw volumes at respective hours. The % inhibition of paw edema was calculated using the formula

% inhibition= (Vc-Vt/Vc) x 100

Where Vc= Paw volume of control, Vt= Paw volume in treated groups.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS version 19.0. Data was expressed as the mean ± SEM. The data was statistically analyzed by using one way analysis of variance (ANOVA) followed by Post hoc- Tukey’s test. Data were considered significant if the p values were below 0.05 ($p < 0.05$).

RESULTS

Synthesis and Characterisation of Silver nanoparticles

Phyto- stabilized Silver nanoparticles were synthesized using the aqeous extract of Black pepper, wherein the alkaloids act as the reducing and capping agents. SEM analysis revealed that the synthesized nanoparticles were 40-100 nm in size, with spherical & cuboidal shape.

Acute Toxicity

In this 14 days period of acute toxicity evaluation, rats given phytostabilised silver nanoparticles at 300mg/kg i.p did not show any treatment related mortality, abnormal clinical signs or remarkable body weight changes (Table 1). On necropsy, no gross pathological observation was recorded in all the experimental animals(Table 2). Thus the LD_{50} of the synthesized silver nanoparticles was greater than 300mg/kg body weight classified under GHS hazard category 5.

Table 1
Individual animal body weight

Step/Dose	Animal number	Body weight (g)		
		Day 0	Day 7	Day 14
I (300 mg/kg b.wt.)	1	167.4	175.6	188.6
	2	160.4	179.8	186.4
	3	164.6	174.6	189.2
I (300 mg/kg b.wt.)	4	195.0	204.2	212.8
	5	199.8	208.6	213.6
	6	199.6	202.4	214.8

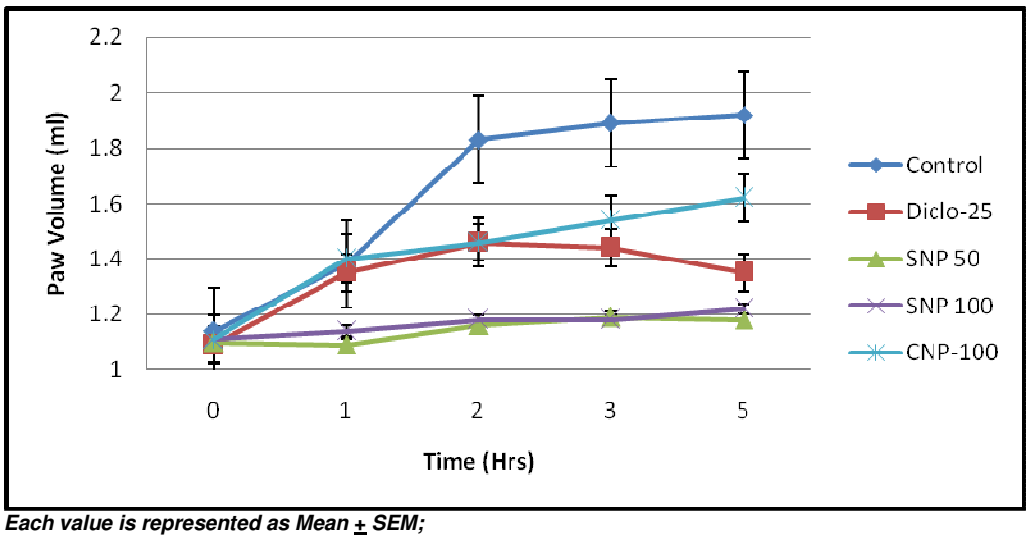
Table 2
Individual animals gross pathological observation

Step/Dose	Animal Number	Organs	Observations
I (300mg/kg b.wt.)	1	Skin, eyes, brain, lungs, heart, liver, kidney, adrenals, spleen and sex glands	No abnormality detected
	2		No abnormality detected
	3		No abnormality detected
II (300mg/kg b.wt.)	4		No abnormality detected
	5		No abnormality detected
	6		No abnormality detected

Carrageenan induced Paw Edema

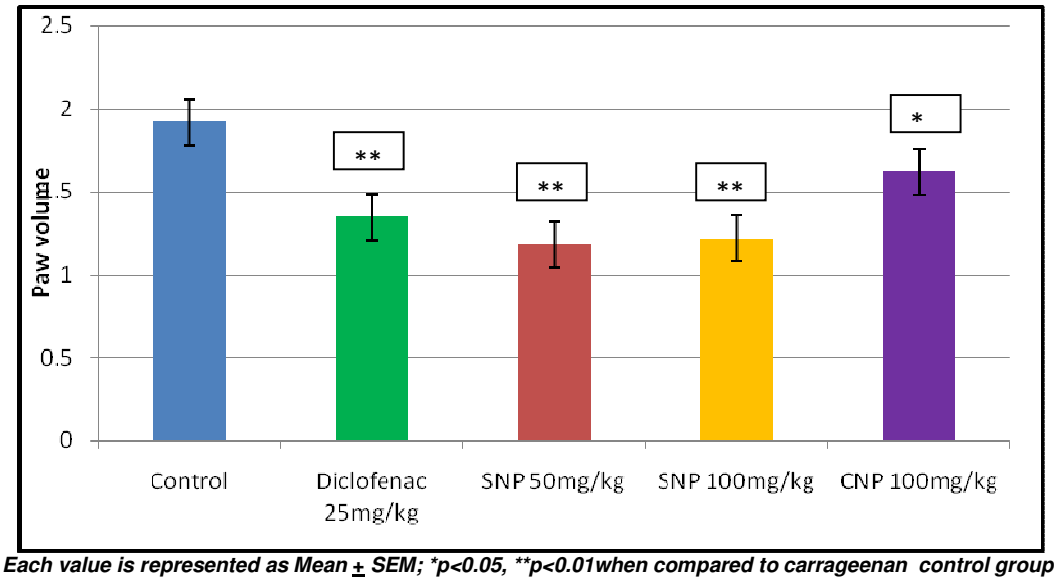
The injection of carrageenan into the right hind paw of wistar rats resulted in a progressive swelling and edema which reached a maximum at 5 hrs. The phyto-stabilized silver nanoparticles caused significant inhibition of paw volume in the carrageenan induced paw edema in rats. The inhibitory effects were also compared with the commercial silver nanoparticles. The decrease in paw volume of the treated groups with respect to time is presented in Fig1. At the end of 5 hrs, the rats treated with phytostabilised silver nanoparticles (SNP 50 and SNP 100) showed significant decrease in paw edema when compared to the controls (Fig 2). The percentage inhibition of paw edema of the phyto stabilized silver nanoparticles (SNP) and the commercial silver nanoparticles (CNP) are presented in Table.3. The phytostabilised silver nanoparticles also showed statistically significant inhibition when compared to commercial silver nanoparticles ($p<0.01$).

Figure 1
Effect of phyto-stabilised silver nanoparticles on carrageenan induced paw edema in rats



Each value is represented as Mean ± SEM;

Figure 2
Effect of phyto-stabilised silver nanoparticles on carrageenan induced paw edema at the end of 5 hrs.



*Each value is represented as Mean ± SEM; *p<0.05, **p<0.01when compared to carrageenan control group*

Table 3
% Inhibition of paw edema at various time intervals

TIME (Hours)	% inhibition of Paw edema			
	GROUP-B Diclo 25mg/kg	GROUP-C SNP-50mg/kg	GROUP-D SNP 100mg/kg	GROUP-E CNP100mg/kg
1	1.84	26.90*	20.90*	1.66
2	25.40*	59.51**	55.24**	25.31*
3	31.52*	59.82**	60.57**	22.80*
5	42.50**	62.50**	57.75**	18.39*

*Significance level * p<0.05 , **p<0.01 compared to control group*

It can be seen that silver nanoparticles at 50 mg/kg concentration showed significant inhibition of paw edema at 2 , 3 and 5 hrs when compared to the control group. It can also be seen that anti inflammatory effect of silver nanoparticles started at 1 hour and peaked at 5 hrs and the maximum inhibitory effect was seen between 2 and 3 hours.

DISCUSSION

Carrageenan-induced rat paw edema is a suitable animal model for evaluating the acute anti-inflammatory effect of test compounds[8]. Carrageenan induces inflammation with the release of many inflammatory and mediators like prostaglandins, leukotrienes, histamine and bradykinin[9]. In the carrageenan induced rat paw edema model, the phytostabilised silver nanoparticles was given by intraperitoneal route at 50 and 100 mg/kg body weight. The intraperitoneal route was selected as the silver nanoparticles are known to have less oral bioavailability. In a previous study by Park *et al* orally administered Ag NPs had a bioavailability of 1.2% in rats treated with 1 mg kg^{-1} Ag NPs and 4.2% in animals treated with 10 mg kg^{-1} Ag NPs[10]. The dose of 50 mg/kg and 100 mg/kg body weight were fixed based on results of in-vitro studies and also based on literature review. The anti inflammatory activity of the phytostabilised silver nanoparticles is attributed to both silver as well as the capping phytochemicals. Piperine ia a major alkaloid present in Black pepper which helps in reducing and stabilizing the silver nanoparticles and is also found to possess anti inflammatory properties[11]. Studies by Jun Soo Bang *et al*

have demonstrated that Piperine showed significant inhibition of carrageenan induced rat paw edema at a dose of 100mg/kg body weight oral dose[12]. In a previous study by L.David et al, silver nanoparticles were synthesised using European black elderberry (*Sambucus nigra – SN*, Adoxaceae family)and the pre-administration of the synthesized silver nanoparticles reduced the paw edema and also the cytokines levels in the paw tissues, early after the induction of inflammation[13]. In a peritoneal adhesion model, silver nanoparticles reduced inflammation in peritoneal adhesions without causing any significant toxic effects[14]. Similarly in a murine model of ulcerative colitis silver nanoparticles showed potent anti inflammatory activity at a dose of 4 mg/kg intracolonically or 40 mg/kg orally[15]. The anti inflammatory effect was brought about by a reduction in expression of matrix metalloproteinase (MMP)-9, TNFα, IL1β and IL-12. In our study, the silver nanoparticles synthesized using Black pepper extract showed significant inhibition of the paw edema at 2, 3 and 5 hrs. The % inhibition of paw edema was estimated to be 62.50 at end of 5th hour. The p value was <0.001, which indicates significant reduction in inflammatory reaction. As the % inhibition was statistically significant at the initial(<2 hrs) and later hours (3-5 hrs) of induction it proves that the phyto stabilized silver nanoparticles synthesized using Black pepper extract inhibit histamine and kinins in the first phase and prostaglandins in the second phase. Moreover the anti inflammatory activity of the synthesized silver nanoparticles as estimated using the carrageenan model was better that the commercial silver nanoparticles (chemically synthesized), thereby proving the synergistic effect of silver and the phytochemicals that cap and stabilize the silver nanoparticles.

CONCLUSION

In the present study, the phytostabilised silver nanoparticles were assessed for acute toxicity and LD50 values were found to be >300mg/kg given intraperitoneally. The anti inflammatory activity of the phyto stabilized silver nanoparticles synthesized using black pepper extract was evaluated using carrageenan paw edema model in rats. The synthesized silver nanoparticles showed significant inhibition of paw edema when compared to the controls and also showed better inhibition compared to the commercial silver nanoparticles. The enhanced anti-inflammatory property of the phytostabilised silver nanoparticles is due to a synergistic effect of both silver and the capping phytochemicals.

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